Recent advances in the development of nitric oxide-releasing biomaterials and their application potentials in chronic wound healing

Min Wu, a Zhihui Lu,a Keke Wu,a Changwoo Nam,*b Lin Zhang* a and Jinshan Guo* a

Chronic wounds, such as pressure ulcers, vascular ulcers and diabetic foot ulcers (DFUs), often stay in a state of pathological inflammation and suffer from persistent infection, excess inflammation, and hypoxia, thus they are difficult to be healed. Nitric oxide (NO) plays a critical role in the regulation of various wound healing processes, including inflammatory response, cell proliferation, collagen formation, antimicrobial action and angiogenesis. The important role of NO in wound healing attracts intensive research focus on NO-based wound healing therapy. However, the application of NO gas therapy needs to resolve the intrinsic shortcomings of gas therapy, such as short storage and release times as well as temporal and spatial uncontrollability of the release mode. So far, various types of NO donors, including organic nitrates (RONO2), nitrates (RONO), S-nitrosothiols (RSNOs), nitrosamines, N-diazeniumdiolates (NONOates), and metal–NO complexes, have been developed to solidify gaseous NO and they were further encapsulated in or conjugated onto a variety of biomaterial vectors to develop NO delivery systems. NO synthetic enzyme mimics to catalyze the production and release of NO from L-arginine have also been developed. This paper reviews recent advances of NO donors, biomaterial vectors, thus-formed NO delivery systems, as well as recently emerged NO synthetic enzyme mimics. Furthermore, this review also summarizes the functions of NO releasing biomaterials that would benefit chronic wound healing, including antibacterial properties and the promotion of angiogenesis, as well as the convenient combination of light/thermal induced NO release with light/thermal therapies, and the prospects for future developing trends in this area.

a Department of Histology and Embryology, NMPA Key Laboratory for Safety Evaluation of Cosmetics, School of Basic Medical Sciences, Guangdong Provincial Key Laboratory of Bone and Joint Degeneration Diseases, The Third Affiliated Hospital of Southern Medical University, Southern Medical University, Guangzhou, China. E-mail: jsguo4127@smu.edu.cn, zlilyzh@smu.edu.cn; Tel: +86-20-61648222
b Department of Organic Materials and Fiber Engineering, Jeonbuk National University, 567 Baekje-daero, Deokjin-gu, Jeonju-si, Jeollabuk-do 54896, Republic of Korea. E-mail: cun120@jbnu.ac.kr

Min Wu is a graduate student in the Department of Histology and Embryology, School of Basic Medical Sciences at Southern Medical University in Guangzhou, China, supervised by Prof. Jinshan Guo. Her research is focused on polymeric scaffolds and tissue adhesives for wound healing applications.

Jinshan Guo obtained his PhD degree in Changchun Institute of Applied Chemistry at the Chinese Academy of Sciences in 2011. After graduation, he went to the University of Texas at Arlington, and then Pennsylvania State University and Harvard University for postdoctoral research. Dr Guo joined the School of Basic Medical Sciences, Southern Medical University as a full professor. Guo’s research focuses on the methodology of biomedical polymers and biomaterials development and their application in tissue regenerative medicine as tissue adhesive and orthopedic biomaterials.
1. Introduction

Wound healing is a complex process involving hemostasis, inflammation, proliferation and tissue remodeling. However, not all wounds heal in a normal period of time. Some wounds develop into chronic wounds, in which the injured tissue stays in a state of pathologic inflammation, leading to protracted and incomplete healing, even non-healing. The most common chronic wounds include pressure ulcers, vascular ulcers, and DFUs, with DFUs as the representative and most intractable chronic wounds. Diabetes is the main contributor to diagnosed chronic wounds. The population of adult diabetic patients has reached 450 million worldwide, almost 6% of the total population. Diabetic patients are prone to develop DFUs, which are estimated to occur in 15% of all diabetic patients. DFUs have a poor prognosis, bring endless pain and substantial economic burden to the patients, and often lead to amputation. The main biological effects of nitrates, such as amyl nitrite (Fig. 1B), isosorbide mononitrate and glyceryl trinitrate, representing the oldest category of NO donors, have been widely used as vasodilators and the treatment of angina pectoris. Amyl nitrite, the first NO donors introduced by T. L. Brunton as early as 1870, was developed for the treatment of patients with coronary artery disease. The main biological effects of nitrates and nitrites are ascribed to the formation of NO. In general, organic nitrates and nitrites can be readily synthesized by reacting alcohols with nitric acid/nitrous acid or other nitrating/nitrosating agents (Fig. 1A). The release of NO from organic nitrates and nitrites can be triggered via several enzymatic and non-enzymatic pathways, including xanthine oxidoreductase (XOR), deoxygenated myoglobin (deoxy-Mb), ascorbic acid, polyphenols and protons.

Nitric oxide (NO) is an endogenous gasotransmitter, which plays a central role in the regulation of wound healing processes including inflammatory response, antimicrobial action, cell proliferation, collagen formation and angiogenesis. NO is endogenously generated from the terminal guanidine moiety of l-arginine catalyzed by three different forms of nitric oxide synthases (NOSs), namely neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS). The constitutive isozymes nNOS and eNOS are expressed in vascular endothelial cells and neurons respectively. The third isozyme, iNOS, is generated only in response to acute inflammatory stimuli. It is generally believed that nNOS and eNOS are essential for maintaining normal physiological homeostasis, and iNOS is related to injury healing. In the inflammatory stage, NO is involved in the immune response regulation, and possesses a wide-ranging antibacterial activity. In the proliferative stage, keratinocyte proliferation at the wound edge is iNOS-dependent, the re-epithelialization is also NO-dependent. NO can promote the migration and proliferation of fibroblasts, which have an important role in collagen production and deposition for wound healing. Furthermore, NO can stimulate the migration and proliferation of endothelial cells, and thus plays a key role in angiogenesis. Declined endogenous production of NO caused by large-area infection and insufficient blood supply, especially in chronic wounds, has been proved to be linked to impaired wound healing. Therefore, supplementation of NO at the wound site represents a promising treatment strategy.

Although NO gas therapy is promising in wound healing, the burst release and uncontrollable delivery of NO greatly limits its applications. To solve this problem, researchers have developed different NO donors, which were loaded into or conjugated onto different biomaterial vectors, to develop a series of NO-releasing biomaterials for biomedical applications. Mimicking the function of naturally occurring NO synthetic enzymes, various NO synthetic enzyme mimics has also been developed, to catalyze the production and release of NO from l-arginine. In this review, we will summarize the recent progresses in the development of different NO donors, NO-releasing biomaterials, and NO synthetic enzyme mimics, and highlight their application potentials in wound treatment, especially in chronic wound treatment (Scheme 1).

2. NO donors and biomaterial vectors for NO delivery

2.1 NO donors

Various NO donors, also known as NO prodrugs, which can be categorized into organic nitrates (RONO₂), nitrites (RONO), S-nitrosothiols (RSNOs), nitroamines, S-diazoniumdiolates (NONOates), and metal–NO complexes, have been explored to solidify NO for biomedical applications (Fig. 1). Among NO donors, RSNOs and NONOates represent the two most widely studied categories due to their ability to spontaneously release NO in physiological media.

Organic nitrates and nitrites, such as amyl nitrite (Fig. 1B), isosorbide mononitrate and glyceryl trinitrate, representing the oldest category of NO donors, have been widely used as vasodilators and the treatment of angina pectoris. Amyl nitrite, the first NO donors introduced by T. L. Brunton as early as 1870, was developed for the treatment of patients with coronary artery disease. The main biological effects of nitrates and nitrites are ascribed to the formation of NO. In general, organic nitrates and nitrites can be readily synthesized by reacting alcohols with nitric acid/nitrous acid or other nitrating/nitrosating agents (Fig. 1A). The release of NO from organic nitrates and nitrites can be triggered via several enzymatic and non-enzymatic pathways, including xanthine oxidoreductase (XOR), deoxygenated myoglobin (deoxy-Mb), ascorbic acid, polyphenols and protons.

Sulfur and oxygen are congeneric elements, and the reactivity of the sulphydryl/thiol group is much higher than that of the hydroxyl group; thus the nitrosyl moiety of nitrites can be easily transferred to the sulphydryl group to form S-nitrosothiols (RSNOS). In fact, thiols could also serve as a co-catalyst to trigger the release of NO from furoxans, nitrate, organic nitrite, and other nitro compounds. RSNOs endogenously exist in both tissue and blood, such as S-nitrosocysteine (CysNO), S-nitrosothiol (GSNO) and S-nitrosoalbumin (AlbSNO) (Fig. 1B). It is of note that RSNOs can also be easily synthesized exogenously via the nitrosation of thiol groups on small molecules, peptides or proteins, such as s-cysteine, glutathione or albumin (Fig. 1A). Nitrosation of inorganic nitrites (NO₂⁻) in an acidic medium is probably the most widely used synthesis approach due to the wide availability of inorganic nitrites and the mild reaction conditions. The facile synthesis of RSNOs makes them one of the most intensively investigated categories of NO donors. The multifunctionality of peptides or proteins also makes peptide or protein based RSNOs easily modifiable, or could be conveniently conjugated onto biomaterials. The release mechanisms of RSNOs include (1) transition metal (e.g., copper ions)-mediated catalytic decomposition,
(2) redox reaction with ascorbate, (3) light or thermal-triggered homolytic cleavage of S–NO bond, and (4) enzyme-mediated release.29,52,54 Due to the low endogenous copper content, photothermal decomposition is the main inducement for RSNO to release NO in biological systems.55 RSNO instability under heat and light may lead to the premature release of NO. Although the instability of RSNOs could be circumvented to some extent, which may bring concerns regarding the long-term storage of RSNO-based materials and obviously limit the practical application of RSNO-based wound dressings in clinical scenarios.7,53

Nitrosamines (N-nitroso compounds) are another class of NO donors, whose nitroso groups could be homolytically or heterolytically transferred to other species. Nitrosamines are formed by the nitrosation of secondary amines with nitrosating agents (Fig. 1A). Although primary amines readily react with nitrosating agents, the formed deamination products are unstable, finally giving diazonium ions (RN$_2^+$). The reactions of secondary amines can be stopped at the nitrosamine stage because there are no necessary R-hydrogen atoms for proton transfer reactions. Most N-nitrosamines at physiological pH in aqueous solution is relatively stable, but is light-sensitive (Fig. 1A).48

$\text{N-Diazeniumdiolates (NONOates)}$ are another type of most widely studied NO donors, which are synthesized via the reaction of secondary amines with high pressured NO gas.56–58 NONOates are considered as a class of very useful NO donors due to their ability to release NO triggered by different stimuli and the possibility of its chemical modification in a highly predictable manner. Efficient NONOates formation needs the help of additional basic residues, such as unreacted amine substrates or metal alkoxide bases, to deprotonate the amine and thus promote its nucleophilic attack by NO (Fig. 1A). The cation (e.g., protonated amines or metals in alkoxide base) can stabilize the anionic charge of the resulting NONOate.59–61 NONOates spontaneously decompose to generate two moles NO per mole of donor with proton-initiation under physiological solution (i.e., 37 °C, pH 7.4).29,62 The NO-release kinetics of NONOates is also affected by environmental factors (e.g., pH, temperature) and the structure of the NO donor precursor (e.g., polyamines) (Fig. 1A).58,63

NO is a powerful ligand of metal ions. The most intensively studied metal–NO coordination compound is sodium nitroprusside (Na$_2$[Fe(CN)$_5$NO], SNP) (Fig. 1B), a widely used vasodilator.64 Although SNP solution is extremely photosensitive, the NO

![Scheme 1](https://example.com/scheme1.png)

*Scheme 1*  The endogenous production and functions of nitric oxide (NO), its solidification to give various NO donors/NO prodrugs, the carrying of NO donors in organic and inorganic biomaterials, and the application of NO-loading biomaterials for wound healing.
release triggered by photolysis under physiological conditions is not significant. In a recent study, another metal–NO coordination compound, Roussin’s black salt ([NH₄][Fe₄S₃(NO)₇]), was used as a NO donor.⁶⁵ The transition metals (such as cobalt⁶⁹ and zinc⁴⁵) on metal–exchanged zeolites could also serve as NO-donors through metal–NO complex formation. NO release from metal–NO compounds requires both light irradiation and single-electron reduction, and the reactions are usually enhanced by thiols.

2.2 Biomaterial vectors for NO delivery

Although NO donors show certain desirable properties in NO solidification, storage and release, their clinical applications are limited by high toxicity, thermal/photo-chemical instability, solidification, storage and release, their clinical applications. Although NO donors show certain desirable properties in NO delivery, their clinical applications are limited by high toxicity, thermal/photo-chemical instability, solidification, storage and release. Therefore, various biomaterial platforms have been developed to carry NO donors and release NO in a sustained and controlled manner. In this section, we will review the NO-releasing biomaterial vectors including organic and inorganic ones (Fig. 2).

2.2.1 Organic vectors. Organic NO vectors mainly include macromolecules, vesicles (including liposomes), dendrimers/branched polymers, polymeric nanoparticles, hydrogels and scaffolds (Scheme 1 and Fig. 2A).

Micelles have been extensively used in the delivery of NO. Hubbell et al.⁶⁶ designed block copolymer pro-amphiphilic and amphiphilic NO-releasing micelles for long-term release of NO. The hydrophobic core of the micelle can protect the NONOate from the influence of water, thereby protecting it against protons required for NO release, resulting in a significantly prolonged release half-life of up to 7 days. Gao et al.⁶⁷ grafted an amphiphilic copolymer, methoxy poly(ethylene glycol)-b-poly(lactic acid) (mPEG-PLA) or 4-tocopheryl polyethylene glycol succinate (TPGS) and nitrate (as a NO donor), on the backbone of poly(2-hydroxyethyl methacrylate) (PEHA), developed a micelle platform for sustained release of NO. TGPS-modified PEHA polymer micelles showed more stable NO release comparing with its counterparts. Kim et al.⁶⁸ designed a dual stimuli-responsive polymeric micelle for NO delivery via the coordination between the diol groups of O₂-protection N-diazoniumdiolate (P-NO) and phenylboronic acid (PBA) moieties on the side chain of a diblock copolymer. The NO-containing micelles can efficiently accumulate at the tumor sites and promote the cytosolic NO release in cancer cells. Ding et al.⁶⁹ reported the self-assembly of micellar nanoparticles formed by a triblock copolymer of poly(ethylene glycol)-b-PNORM-b-poly(ethylene glycol) (PEG-b-PNORM-b-PEG), in which the NO-releasing molecule is a N,N°-dinitroso-o-phenylenediamine (DNP) derivative, capable of releasing NO under light irradiation (Fig. 2(A1)).

Vesicles have been reported as a carrier to encapsulate both gaseous NO and solidified NO donors. Huang et al.⁷⁰ reported the encapsulation of NO into echogenic liposomes (ELIP) can be achieved via freezing using a high pressure technique. NO-containing liposomes (NO-ELIP) can protect NO from being scavenged by hemoglobin and realize effective NO delivery. Suchyta and Schoenfisch⁷¹ reported the adjust of NO release from N-diazoniumdiolate encapsulated liposomes by changing the molecular structure of NO donor and/or the phospholipid composition (independently or in combination). Katayama’s group⁷²,⁷³ prepared NONOate-containing PEGylated liposomes (NONOate-LP) that showed protonation induced retarded the release of NO. Their study successfully demonstrated for the first time that incorporating an NO donor in the PEGylated lipidosome can improve the enhanced permeability and retention (EPR) effect (Fig. 2(A2)). Duan et al.¹⁸ fabricated NO-releasing vesicles synthesized by self-assembly of NO-releasing amphiphiles via direct photoresponsive polymerization of N-nitrosoamine-based NO monomers, for corneal wound healing.

So far, various NO releasing dendrimers/branched polymers have been developed.⁷⁴–⁷⁶ Stasko and Schoenfisch reported for the first time the potential of dendrimers as powerful NO storage/release carriers.⁷⁷ Compared to their small molecule counterparts, the secondary amine-containing dendrimers showed a unique dendritic effect and had a significantly prolonged NO release time. The Schoenfisch group has conducted a series of research studies on NO-releasing dendrimers with enhanced anti-biofilm activity.⁷₈–⁸₅ Katsumi et al.²⁴ designed an S-nitrosylated l-serine-modified polyamidoamine dendrimer (SNO-Ser-PAMAM) and their results showed that SNO-Ser-PAMAM is a promising NO donor for kidneys, and could be used to efficiently prevent renal ischaemia/reperfusion injury. Frost et al.²⁵ modified hyperbranched polyamidoamine (HPAMAM) with S-nitroso-N-acetyl-d-penicillamine,
which was then nitrosated to form a controlled, high-capacity NO-donating compound SNAP-HPAMAM.

Nanoparticles represent attractive materials for the encapsulation of NO-donors to create NO-release systems. Duong et al. reported NO releasing N-diazeniumdiolate moieties crosslinked core nanoparticles, which exhibited slow and controlled release of NO, and showed considerable antibacterial effects. They also used a similar approach to prepare NO and gentamicin co-delivery nanoparticle to reduce the formation of a Pseudomonas aeruginosa biofilm. Ghalei et al. described the fabrication of NO-releasing silk fibroin nanoparticles (SF NPs) using S-nitroso-N-acetylpenicillamine (SNAP) as the NO donor. SNAP-SF NPs exhibited strong antibacterial properties against methicillin-resistant Staphylococcus aureus (MRSA) and Escherichia coli (E. coli). Wu et al. constructed S-nitrosoglutathione (GSNO) functionalized poly(ethylene glycol)-block-poly(propylene sulfide) (PEG-PPS) nanoparticles to deliver doxorubicin (DOX). Such GSNO functionalized nanoparticles could release DOX in a ROS triggered manner and increase the intracellular accumulation of DOX (Fig. 2(A4)).

Seabra et al. incorporated S-nitrosogluthatine (GSNO) into a thermoresponsive hydrogel consisted of poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO–PPO–PEO, Pluronic F127) and chitosan (CS), which exhibited...
antibacterial effects against *Pseudomonas aeruginosa*. Recently, they also described the synthesis, cytotoxicity, and antibacterial effects of alginate hydrogel containing $S$-nitroso-mercaptopheuccic acid (NO donor) and silver nanoparticles (AgNPs).\textsuperscript{96} Park et al.\textsuperscript{97} incorporated $S$-nitrosothiolated gelatin (GelSNO) into injectable gelatin-based hydrogels (GHs) crosslinked by horseradish peroxidase (HRP) and $\text{H}_2\text{O}_2$ (Fig. 2[A5]). The peroxynitrite (ONOO\textsuperscript{-}) was formed in situ to confer effective antibacterial effects. Through a mechanism driven by heat, visible light, or oxidizing agent, NO was released from the GH/GelSNO hydrogels, which showed significant bactericidal effects against both Gram-positive and Gram-negative bacteria. Moreover, Ramadass et al.\textsuperscript{98} reported a silk fibroin-polyvinyl alcohol (SF-PVA) nanofibrous scaffold via type I collagen peptide (CP), and a NO donor, $S$-nitrosogluthathione (GSNO), to treat non-healing diabetic ulcers (Fig. 2[A6]). Electrospun fibers doped with NO donor are presented as widely used macromolecular scaffolds with a great potential for tissue engineering applications.\textsuperscript{99–101}

### 2.2.2 Inorganic vectors

Inorganic NO vectors mainly contain metallic nanoparticles, silica particles, zeolites and metal organic frameworks (MOFs) (Fig. 2B). Recently, metal and metal oxide nanoparticles serve as intensively researched NO-releasing platforms due to their unique physical, chemical, optical, and electronic properties.\textsuperscript{45,102–104} Through the strong coordination between thiol groups of $N$-diazienumdiolate-modified thiol containing molecules with gold (Au) nanoparticles, Schoenfisch and co-workers developed NO releasing gold nanoparticles, which can release NO spontaneously in aqueous media (physiological pH).\textsuperscript{105} Seabra et al.\textsuperscript{106} prepared silver nanoparticles (Ag NPs) and $S$-nitrosogluthathione (GSNO) separately or simultaneously incorporated polymeric films composed of poly(vinyl alcohol) (PVA) and poly(ethylene glycol) (PEG). The PVA/PEG films containing GSNO and/or AgNPs showed a strong antibacterial activity and cytotoxic effect against tumor cell lines. Singh et al.\textsuperscript{107} studied the catalytic and antimicrobial properties of hybrid SNAP-loading zinc oxide (SNAP-ZnO) nanoparticles, which could catalytic release NO under physiological conditions and demonstrate antimicrobial effects.

Silica-based materials are widely used in biomedical area due to the customization of size, morphology, and composition benefited from their facile synthesis.\textsuperscript{101,108–111} Munaweera et al.\textsuperscript{116} reported a NO and cisplatin releasing wrinkle-structured amine-modified mesoporous silica (AMS) nanoparticles, which were prepared via condensation of tetraethylorthosilicate (TEOS) and 3-triethoxysilylpropyl diethylenetriamine (Si-DETA) and subsequently doped with $N$-diazienumdiolate and cisplatin (Fig. 2[B1]). Schoenfisch et al.\textsuperscript{117} prepared NO-releasing mesoporous silica nanoparticles (MSNs) using an aminosilane-template surfactant ion exchange reaction.

To increase the payload of NO and expand the potential applications of NO loading materials in more diverse biomedical areas, porous inorganic materials, such as MOFs and zeolites, have also been used as vectors for NO donors (Fig. 2[B2]).\textsuperscript{118–121} The high NO loading capacity of MOFs and zeolites makes them extremely attractive for the use in biological and medical applications. MOFs provide an NO loading substrate with tunable physical and chemical properties. Wheatley et al.\textsuperscript{122} used zeolite-A, an alternating alumina/silica network with cobalt (Co) cations, to chemisorb NO, and showed that the NO released from Co-exchanged zeolite-A could inhibit the adhesion and aggregation of human platelets \textit{in vitro}. Fox et al.\textsuperscript{43} demonstrated that the NO release capacity of a novel NO-storing Zn\textsuperscript{2+}-exchanged zeolite material and its potent antibacterial properties against both Gram-negative and Gram-positive bacteria. Reynolds et al.\textsuperscript{123} showed that copper-based MOF $\text{H}_2[(\text{CuCl})_3(\text{BTTri})]_3$ (Cu-BTTri, 1,3,5-tris[1H-1,2,3-triazol-5-yl]benzene) could trigger an elevated level of NO released from GSNO, which was incorporated with naturally derived polysaccharide chitosan to form membranes for wound healing applications.

### 2.3 Enzyme mimics promoting the endogenous and exogenous NO generation

As mentioned above, the NO release from NO donors needs the help of triggers, such as thiols, enzymes and so on, which could serve as catalysts to promote NO production from NO donors either \textit{in vivo} or \textit{in vitro}. The use of natural enzymes, such as glutathione peroxidase, can realize the biotransformation of endogenous NO donor to produce NO. Nevertheless, low stability and short shelf life are inherent shortcomings of naturally occurring enzymes.\textsuperscript{124} Various enzyme mimics, such as polymers and hydrogels, MOFs, and metal or metal oxide nanoparticles, had been developed to simulate naturally occurring enzymes, to catalyze the production of NO from L-arginine.\textsuperscript{37,125,126}

The Reynolds group considered that incorporation of copper-based MOFs (Cu-BTTri) into hydrophilic poly (vinyl alcohol) (PVA) or hydrophobic polyurethane (PU) can promote the endogenous NO generation (Fig. 3A).\textsuperscript{127} The obtained Cu-BTTri/PVA films exhibited a higher swelling ratio, thus enhanced the interaction between catalysts and GSNO, resulting in faster NO generation. Zhang et al.\textsuperscript{11} reported a one-step metal–catecholamine assembly strategy to prepare a durable \textit{in situ} NO-generating biomimetic dopamine-Cu(II) ([DA-Cu(II)]) coating. The coating could decompose endogenous $S$-nitrosothiols (RSNOs) in fresh blood, to generate NO \textit{in situ} (Fig. 3B). Zhao et al.\textsuperscript{128} reported a copper-based surface-attached metal–organic framework (Cu-SURMOFs) of copper(II) benzene-1,3,5-tricarboxylate (CuBTC) on the surface of alkali-activated titanium using layer-by-layer (LBL) assembly, for NO \textit{in situ} generation from endogenous $S$-nitrosoglutathione (GSNO) (Fig. 3C). The Chandrawati group\textsuperscript{19} discovered that zinc oxide (ZnO) particles could mimic the activities of $\beta$-galactosidase and glutathione peroxidase, and catalyzed both exogenous ($\beta$-gal-NONOate) and endogenous (GSNO) NO donors to generate NO. The physiological NO levels could be attained by simply modulating the concentrations of GSNO and ZnO. Li et al.\textsuperscript{37} developed a natural platelets (PLT) liposome loaded with L-arginine and magnetic $\gamma$-Fe$_2$O$_3$ nanoparticles (PAMNs) for thrombus-targeted delivery of L-arginine and \textit{in situ} NO generation (Fig. 3D). Their rapid targeting to stroke lesions as well as \textit{in situ} NO generation enhanced the expansion of blood vessels and reduced platelet aggregation, thus delayed thrombosis plaques development.
Fig. 3 (A) Structure of Cu-BTTri and the cumulative NO release from GSNO loading Cu-BTTri/PVA membranes. (B) Cu(II) crosslinked mussel-inspired adhesive coating and the calculated release rates of NO at different Cu(II) concentrations. (C) The layer-by-layer (LBL) deposition of CuBTC coating on alkali-activated titanium surface and the representative real-time NO generation. (D) The fabrication of PAMNs by the extrusion method and the photomicrographs of NO production over time in bEnd.3 cells.

Fig. 4 (A) NO-releasing mPEG–PLGA nanoparticles promotes angiogenesis. (B) NO@HKUST-1/PCL/Gel (NO@HPG) scaffold promotes diabetic wound healing. (C) NO released from PCL/CS–NO dressings switching on/off by β-glycosidase improves wound healing. (D) Hydration-controlled NO release from PAA:F127/GSNO topical hydrogels promotes wound healing.
Recently, several redox systems have been employed to in situ produce NO from nitrite. L-Ascorbic acid could serve as a reducing agent to reduce nitrite into NO. The presence of copper complexes as a catalyst was proved could increase and prolong NO generation when compared to that of nitrite and L-ascorbic acid alone.129 Amal’s group found that Fe(II) ions could also serve as a reducing agent to reduce nitrite into NO.129–131

3. Wound healing applications of biomaterial NO-releasing systems

In recent years, the use of NO gas therapy for wound healing has been extensively and intensively studied. Endogenous NO mediates a lot of important biological processes occurring after cutaneous injury, including inflammatory response, cell proliferation and collagen formation. Moreover, NO plays an important role in killing microbes and promoting angiogenesis, to accelerate tissue regeneration and wound healing. Due to the importance of NO in wound healing and the inefficient endogenous NO produce especially in chronic wound where blood supply was mostly destroyed, different NO-releasing biomaterials have been designed to deliver NO for promote chronic wound healing.

3.1 NO releasing biomaterials promote angiogenesis

NO-releasing materials can promote injured tissue regeneration by enhancing angiogenesis, collagen deposition, and reepithelialization.132–136 Yang et al.137 reported the feasibility of methoxy poly(ethylene glycol)-b-poly(lactic-co-glycolic acid) nanoparticles (mPEG–PLGA NPs) as NO-releasing materials could induce enhanced angiogenesis, representing a promising therapy for wound healing and treating hind limb ischemia (Fig. 4A). Xu et al.20 developed a copper-based MOF HKUST-1 as a NO-loading vehicle and with core–shell structure through electrospinning (Fig. 4B). The obtained NO sustained release system could promote endothelial cell growth and significantly improve angiogenesis and collagen deposition in the wound bed, and also exhibited anti-inflammatory properties, thus eventually accelerated diabetic wound healing. Zhao et al.44,135 prepared a novel functional wound dressing by combining electrospin poly(ε-caprolactone) (ε-PCL) nonwoven mat with glycosylated NO compound gratted chitosan (CS–NO) as NO-releasing biomaterials switched on/off by β-glycosidase (Fig. 4C). The PCL/CS–NO dressing exhibited good stability under physiological conditions and could release NO in a controllable and sustainable manner under the catalysis of galactosidase. Results showed that the sustained release of NO accelerated wound healing in comparison with control and
PCL/CS groups. Oliveira et al. developed a supramolecular interpolymer complex hydrogels comprising with F127 (poly-ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol), PEG–PPG–PEG micelles embedded in a poly (acrylic acid) (PAA) matrix, with GSNO molecules being distributed in the hydrophilic domain (Fig. 4D). A preliminary in vivo study on full-thickness excisional wounds on mice showed that the topical NO release from the PAA:F127/GSNO hydrogels could be triggered by the absorbed exudate and lead to enhanced angiogenesis and more organized collagen fiber deposition.

3.2 NO releasing biomaterials exhibit antibacterial ability
Wound healing is often accompanied by infection. NO has antimicrobial ability due to the combination of nitrosative and oxidative mechanisms. After reacting with oxygen and superoxide, NO transforms into dinitrogen trioxide and peroxynitrite, respectively. Dinitrogen trioxide causes DNA deamination, while peroxynitrite induces lipid peroxidation and membrane damage. NO was proved exhibiting dose-dependent antibacterial activity against biofilms. As a diatomic free radical, NO gas possesses preferable antimicrobial capability, beneficial for wound healing applications. Lee et al. developed an in situ hydrogel-forming/NO-releasing powder dressing (NO/GP) possessing the benefits of both powders and hydrogels (Fig. 5A). The results of an in vitro antibacterial study demonstrated that NO/GP would be a promising alternative to dressings for the treatment of infected wounds. Due to the abuse of antibiotics and the increase of multidrug resistant bacteria, Huang et al. reported a photothermal ingredient loading β-cyclodextrin-functionalized graphene oxide (GO) NIR light responsive nanovehicles combined with the NO donor BNN6 in a GelMA/HA–DA hydrogel for bacteria-infected wound healing. The results revealed that hydrogel was an ideal antibacterial material that could improve collagen deposition and angiogenesis, thus promote wound healing (Fig. 5B). Chitosan is also used for wound healing due to its antimicrobial and anti-biofilm effects. Yoo et al. developed NO-releasing films (CS/NO film) composed of chitosan (CS) and GSNO as a NO donor used for the treatment of MRSA biofilm-infected wounds under diabetic condition (Fig. 5C). Yu et al. grafted a three generation dendritic poly(amidoamine) (PAMAM-G3) onto a polydopamine (PDA) coated iron oxide nanocomposite (Fe3O4@PDA), and subsequently loaded NO with the formation of NONOate. The thus-obtained Fe3O4@PDA/PAMAM@NONOate exhibited controllable NO release upon 808 nm laser light irradiation, which also showed excellent bacteria-separation efficiency (Fig. 5D). A recent study showed an increased and extended NO release from SNAP when combined with cerium oxide nanoparticles (CNP), due to the preservation of the NO donor by CNP, the synergistic effect between NO donor and CNP enhanced the antibacterial effect.

3.3 Synergetic therapy strategies of NO releasing biomaterials
Recently, many near-infrared (NIR) responsive NO release platforms were developed, the NO release from which can be effectively triggered by NIR light. Therefore, the photothermal-responsive NO release platform represents a smart gas release...
strategy with good therapeutic effects. Gao and his team report a new near-infrared 808 nm laser-mediated NO-releasing nano-vehicle (MoS2-BNN6) through simple assembly of β-cyclo-dextrin (β-CD) modified MoS2 nanosheets (MoS2-β-CD) with a heat-sensitive NO donor N,N'-di-sec-butyl-N,N'-dinitroso-1,4-phenylenediamine (BNN6) for safe, rapid, and effective disinfection of inflammatory wounds (Fig. 6A).\textsuperscript{153} When exposed to 808 nm laser irradiation, hyperthermia from MoS2-BNN6 can precisely control NO delivery and release, exhibited high antibacterial activity against AmpR \textit{coli} and \textit{E. faecalis}. The synergetic antibacterial strategy based on NIR photothermally responsive MoS2-BNN6 can perturb and damage cell membranes structure for rapid and highly effective killing of bacterial (Fig. 6B). Han \textit{et al}. constructed an intelligent NO nanogenerator triggered by NIR light \textit{via} encapsulated the photothermal sensitive sodium nitroprusside (SNP) inside the MOF that can specifically recognize and adhere to Gram-negative bacteria and depolarize the bacterial membrane to increase permeability (Fig. 6C).\textsuperscript{154} The synergistic antibacterial effects can achieve precise treatment of bacterial infections and promote wound healing without damaging normal tissues, and thus has great clinical application potential (Fig. 6D).

4. Conclusion and outlook

Due to the critical role of nitric oxide (NO) in the regulation of wound healing processes including inflammatory response, antimicrobial action, cell proliferation, collagen formation and angiogenesis, NO therapy has great application potential in wound healing, especially in chronic wound healing. To prolong NO release time and improve NO release controllability, NO gas was solidified to give various NO donors/NO prodrugs, including organic nitrates (RONO\textsubscript{2}), nitrites (RONO), N-nitrosothiols (RSNOs), nitrosamines, N-diazoniummidolates (NONOates), and metal–NO complexes, and NO donors were further loaded into various organic and inorganic biomaterial vectors to develop NO delivery systems. The developed NO-releasing biomaterials could release NO when exposed to different stimuli, such as protons, metal ions, enzymes, heat and light irradiations, and light/thermal induced NO release could be conveniently combined with light/thermal therapies in biomedical applications. To realize more convenient NO delivery, NO synthetic enzyme mimics based on polymers, MOFs, metal or metal oxides, catalysing the \textit{in situ} production of NO from l-arginine were also developed. NO releasing biomaterials are beneficial in killing microbes and promoting angiogenesis, to accelerate tissue regeneration and wound healing, thus have great application potentials in chronic wound healing.

However, the exact role of NO in wound healing is still controversial, and the exposure dose and duration of NO effective for wound healing are closely related to the microenvironment, cell type, and animal model. In this respect, more attention should be taken to these factors in the design of NO-delivery systems as a wound healing therapy. In addition, the releasing characteristics of NO-releasing biomaterials should also be carefully designed and evaluated to meet the requirements of clinical applications, especially for chronic wound healing. On the other hand, along with the deepened understanding of NO related biology and the progress of material science, we believe that more NO synthetic enzyme mimics and \textit{in situ} NO producing nano/macro-reactors or scaffold-based NO mini-factories would be developed in the future to realize more convenient NO production and environmental-responsive release for chronic wound healing and smart management.

Author contributions

Min Wu: writing, software; Zihui Lu: figures drawing; Keke Wu: revise; Changwoo Nam: conception, revise; Lin Zhang: revise; Jinshan Guo: conception, supervision, edit.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The project was supported by the National Natural Science Foundation of China (82073417, 81872514), the Joint Foundation for Basic and Applied Basic Research Project of Guangdong Province (Grant no. 2020A1515110602) and the Open Research Fund of State Key Laboratory of Polymer Physics and Chemistry, Changchun Institute of Applied chemistry, Chinese Academy of Sciences.

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